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Identification and Varietal Response of Soybeans to Soybean Mosaic Virus and Tobacco Ringspot Virus.

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IDENTIFICATION AND VARIETAL RESPONSE OF SOYBEANS TO SOYBEAN MOSAIC VIRUS AND TOBACCO RINGSPOT VIRUS

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ABSTRACT

Two Louisiana virus isolates were identified by symptomatology, host range, physical properties, transmission and serology as soybean mosaic virus (SMV) and tobacco ringspot virus (TRSV).

The symptoms of each virus were described on soybeans (Glycine max (L.) Merr.) and other hosts. Temperatures had a marked influence on the intensity of these symptoms in soybeans. At 20 C symptoms were more severe in test plants than in plants grown at 25 C or higher.

SMV was transmitted in 30 percent of the seed of Dare soybeans and to lesser extents in other varieties. TRSV was transmitted at a high rate in all varieties and as much as 93 percent in Hill.

In greenhouse tests where all inoculated plants were observed to be infected, SMV significantly reduced the yields of Lee, Dare, Hill and Semmes varieties. The data suggested that Davis and Bragg may possess tolerance to SMV. TRSV significantly reduced yields of all varieties tested.

Under field conditions where the extent of infection was not known, the yields of all varieties were not significantly reduced by inoculation with SMV. The yields of the varieties Davis and Semmes were significantly reduced by TRSV.

SMV failed to react with antisera in either microprecipitin or agar diffusion tests. TRSV formed distinct precipitin zones when allowed to react with antisera to Louisiana, Arkansas and North Carolina isolates of the virus.

Ceratoma trifurcata Forst. (bean leaf beetle) failed to transmit either SMV or TRSV from infected to healthy Dare soybean plants. *Myzus persicae* Sulz. (green peach aphid) transmitted SMV from infected to healthy Dare plants, but did not transmit TRSV in similar tests.

INTRODUCTION

In recent years, soybeans (Glycine max (L.) Merr.) have greatly increased in importance as a commercial crop in the United States. Soybeans are widely used as a protein source in many animal feeds and research is currently underway to develop high protein synthetic foods from soybeans. Soybean oils are used in several manufacturing processes, including the plastics industry. In response to this increased demand, U.S. soybean production has approximately doubled in the last ten years from 555 million bushels in 1960 to an estimated 1,134 million bushels in 1970 (49).

Soybean production has also increased sharply in Louisiana in recent years. In 1970, 38 million bushels were harvested from 1,688,000 acres in Louisiana. This represented an estimated income of over $108,200,000 to Louisiana farmers and made soybeans the most important cash crop in the state (50).

Soybean plants are subject to many disease inducing agents, including fungi, bacteria, nematodes and viruses, which cause an estimated annual loss of 12 percent of the total crop (11).

Although soybean virus diseases in Louisiana are not yet of major importance, surveys of many soybean fields indicated that virus diseases were present in all areas of the state. Three different viruses appear to be most common. These were identified by symptomatology as soybean mosaic virus (SMV) (7), bean pod mottle virus (BPMV) (40), and tobacco ringspot virus (TRSV) (1). The two viruses investigated in this study were SMV and TRSV.

SMV causes a mosaic pattern on emerging trifoliate leaves followed by a downward curling of the leaf margin giving a distorted, oak-leaf-shaped appearance to the leaves. As the leaves become older, they develop a blistered appearance along the main veins. Infected plants are generally somewhat reduced in size and produce fewer seeds than normal plants. SMV is transmitted through seed (25) and is spread from plant to plant in the field by several species of aphids (7).

TRSV causes a disease commonly called bud blight of soybean (1). Many strains of the virus exist and symptoms may be quite variable. In general, symptoms include mottling of the young trifoliate leaves, a rusty flecking on the youngest leaves and a bronzing and downward curving of the stem tip. Soybean plants infected with TRSV may be severely stunted, depending on variety, and often remain green after healthy plants have matured. Seed production is always reduced. TRSV is transmitted through seed (10), but no important insect vector has been found for soybeans.

This study was designed to positively identify the two isolates of SMV and TRSV by using symptomology, host range, physical properties, transmission and serology.

The second objective was to investigate the reactions of six Louisiana soybean varieties to SMV and TRSV. Field and greenhouse tests were conducted to determine percent of seed transmission and effects on yields of each variety.

REVIEW OF LITERATURE

A number of viruses which affect soybeans have been reported in the literature (22, 27). These viruses include yellow stipple (51), red node (46), yellow bean mosaic (32), alfalfa mosaic (2, 45). tobacco streak (13), bean pod mottle (40), cowpea mosaic (19), soybean mosaic (6), and tobacco ringspot (1). The two of most economic importance nationally are probably SMV and TRSV. In Louisiana SMV and BPMV have been observed most frequently.

SMV occurs to a limited extent in all soybean producing areas of the U.S. and varies in severity in different areas and on different varieties (11). Kendrick and Gardner (25) reported yield reductions of 30-75 percent in Indiana during 1921 and 1922. Soybean varieties, however, have been considerably improved since that time. In 1968, Ross (36) reported yield reductions of 8-25 percent on Hill and Lee soybeans in North Carolina.

SMV was first reported in 1916 by Clinton (6) who observed it on several varieties of soybeans in Connecticut. Gardner and Kendrick (16) in 1921 established the virus nature of the disease by seed transmission, grafting and mechanical inoculation. They described symptoms which included distinct mottling and a downward rolling of the leaf edges. Conover (7) observed symptoms which included vein clearing followed by a yellowish mottle, formation of puffy areas along the major veins of the leaf and leaf margins which were curved downward at the sides.

Observations that SMV was more severe in certain parts of the growing season led some investigators to suggest that symptomatology of the disease was correlated with temperature. Johnson (24) in 1922 reported that high temperatures suppressed symptoms of SMV. Conover (7) established that SMV symptoms were very severe at 18.5 C, but were mild or not visible at 29.5 C. Comparable results were obtained by Walters (52) in 1963 with temperatures of 20 and 27 C.

SMV has a narrow host range and for many years soybean was considered to be the only systemic host. In 1948, Conover (7) inoculated a wide range of leguminous and nonleguminous plants with SMV, but failed to produce infection. Galvez (15), in 1963, found that SMV systemically infected and produced symptoms on Cassia occidentalis L., Phaseolus lathyroides L. and Sesbania exalta (Raf.) Cory. Walters (52) reported Canavalia ensiformis (Jacq.) O. Ktac., Cyamopsis tetragonoloba L., Lespedeza stipulaca Maxim., Lupinus albus L., and Phaseolus lunatus L. var. Henderson Bush Lima to be hosts for SMV. According to Ross (34), Phaseolus vulgaris L. var. Kentucky Wonder Pole Wax is a local lesion host for SMV.

SMV has been transmitted mechanically (7, 16, 32), by several species of aphids including the green peach aphid (Myzus persicae Sulz.) (7, 15) and by seed (7, 16, 25, 32).

SMV in extracted sap has been reported to have a dilution end point of 10^{-3}-10^{-5}; a thermal inactivation point of 58-66 C; and a survival in vitro of 1-4 days at room temperatures (20-22 C) (7, 15, 32, 41).

Galvez (15), in 1963, was one of the first investigators to conduct detailed studies on purification and morphology of soybean mosaic virus. He purified SMV by density-gradient centrifugation and obtained infectious rod-shaped particles 650-725 mu in length and 15-18 mu wide. Brandes and Wetter (5) listed SMV particles as about 750 mu long. Ross (35) purified SMV and obtained flexuous rods 740±10 mu.

TRSV occurs mainly throughout the midwestern U.S. and southern Canada, although it has been found in most soybean producing areas (11). Bud blight, the disease caused by TRSV on soybeans, has been sporatic or cyclic in occurrence, causing heavy losses of 25-100 percent in some areas between 1943 and 1947 and slacking off from 1949 through 1954 (20). The disease was again very serious in the midwest during 1955-57, but was practically nonexistant in 1958 (3, 12).

TRSV was first described by Fromme and Wingard (14) in 1922, but it was not until 1934 that it was experimentally shown to infect soybeans (31). The disease was first observed in nature by Samson (39) in 1941 in plots of vegetable soybeans in Indiana. Melhus (29) observed TRSV in Iowa in 1942 and Johnson (21) reported it in Ohio in 1943. In 1946, Allington (1) verified earlier identifications of the virus by thermal inactivation tests, cross-protection and symptomatology. He named the disease "bud blight" because of the bronzing, curling, brittleness and necrosis of the apical growing point. Hilderbrand and Koch (18) observed a yellow discoloration of young leaves with a tendency toward rugoseness, rolling, vein clearing and necrotic stippling in addition to the symptoms described by Allington.

Temperature seems to have a definite effect on symptom expression of TRSV. Khan and Latterell (26) demonstrated that plants grown at temperatures below 26 C expressed strong bud blight symptoms and were severely stunted.

TRSV has an extremely wide host range, including many cultivated and native species (12, 31, 38, 41, 47, 48). Tobacco (Nicotiana tabacum L.) and cowpea (Vigna sinensis Torner var. Early Ramshorn) are often used to assay for TRSV. Most varieties of tobacco will produce characteristic local and systemic "ringspots" when inoculated with TRSV (41). Early Ramshorn cowpea produces brown to purplish ringspot lesions on inoculated leaves followed by systemic invasion of the stem tip and subsequent death of the seedling (41).

TRSV has been transmitted mechanically (3, 31), through soybean seed (3, 10) and by nematodes (28). No important insect vectors have been reported, but the fact that the disease usually appears first at the border of a field and progresses inward suggests that there are insect carriers of the virus (11).

TRSV in extracted sap has been reported to have a dilution end point of 10^{-3}; a thermal inactivation point of 55-66 C; and a survival in vitro of 5-6 days at room temperatures (31, 41).

Preparation of partially purified TRSV was reported by Stanley (43) in 1939. Since then, this virus has been described by many other investigators. Steere (44) and Stace-Smith et al. (42) described the particles as hexagonal in shape and about 25 mu in diameter. Corbett and Roberts (8) reported the virus particles to be icosahedral shaped and 28 mu in diameter.

MATERIALS AND METHODS

Source of Inoculum

The SMV strain used in this study was isolated from a Dare seedling from commercial seed grown in the greenhouse. It was similar in severity of symptom expression to other isolates that were collected from various soybean fields throughout the state. TRSV was obtained from a Dare seedling the seed of which was obtained from a TRSV infected Curtis plant at Bayhill, Louisiana. Both isolates were maintained in young Dare plants in the greenhouse throughout this research. Soybean seed used in greenhouse and field tests were obtained from the Alexandria Seed Company, Alexandria, Louisiana.

Method of Inoculation

All inoculations, unless otherwise stated, were made by first dusting leaves with carborundum (silicon carbide powder-600 mesh) then rubbing them with the thumbs which were dipped in infective sap. The inoculum was the expressed sap from diseased soybean leaves which were ground with a mortar and pestle and to which was added an equal volume of 0.01 M phosphate buffer of pH 7.2, then strained through cheesecloth.

Infectivity Assay

Dare soybeans were used to assay the infectivity of SMV and cowpea (*Vigna sinensis* Torner "Early Ramshorn") for TRSV.

Host Range Studies

Plants used in host range studies were grown in an air conditioned greenhouse where temperatures ranged from 25-27 C. Inoculations were made either on the cotyledons or the first bifoliate leaves depending on the plant tested. Plants were observed and symptoms were recorded during a 14 day period. Dare plants were inoculated with sap obtained from all assay plants tested, regardless whether or not they had definite symptoms, to determine if any were symptomless hosts for TRSV or SMV.

Influence of Temperature on Symptom Expression

Because of a wide variation of temperatures in the greenhouse during winter and summer (24-41 C), it was desirable to know the effects of temperature, if any, on symptom expression. This was accomplished by inoculating the bifoliate leaves of Dare soybeans and placing the plants immediately in controlled environment chambers (Sherer Mobile Greenhouse, Model MG 8) at specific temperatures after inoculation. The test plants were incubated at 20, 25, 30, 35, 40 C respectively for 14 "daylight" hours daily and all intermittently at 21 C for 10 hours of "night". Ten replications were used in each test. Plants were observed daily and symptoms were recorded as they appeared.

Physical Properties

A. Tolerance to dilution

Infective sap was diluted with distilled water in a range between 10^{0} to 10^{-7}. Dare and Early Ramshorn assay plants were

inoculated with the various dilutions of SMV and TRSV in the greenhouse to determine their tolerance to dilution. Symptoms were recorded as they appeared.

B. Thermal inactivation

Enough sap from the TRSV and SMV infected Dare plants was drawn into 15 thin-walled capillary tubes for each virus to fill half of each of them. One end of each tube was sealed by a flame. The other end of each tube was inserted in a cork. The tubes of sap were then floated in water baths heated to 50, 60, 70, 80 and 90 C respectively for ten minutes. Three tubes of sap were used at each temperature. All samples were stored in an ice bath prior to and after heating. Each replicate was then assayed on Dare and Early Ramshorn plants for infectivity. The entire experiment was repeated twice. After it was determined that the inactivation point lay between 50 and 60 C for SMV and 60 to 70 C for TRSV, another test was run with 5 C intervals between the previous temperatures.

C. Longevity in vitro

Resistance to aging in vitro studies consisted of storing infective sap in stoppered test tubes at room temperature (20-22 C), removing small aliquots daily and testing for infectivity on Dare and Early Ramshorn plants.

Insect Transmission

A. Bean leaf beetle

Bean leaf beetles (Ceratoma trifurcata Forst.), collected from a soybean field, were starved for one hour and then allowed to feed

on TRSV or SMV infected leaf tissue. The insects were placed immediately on healthy Dare soybeans and allowed to feed. If no feeding was apparent within ten minutes, the beetles were removed and others which had fed on infected tissue were placed on the host plants. This procedure was continued until each plant had been fed on once. Plants were then placed in the greenhouse and observed for symptoms. Fifteen replications were used for each virus. The controls were beetles fed on healthy plants, then on test plants.

B. Green peach aphid

Green peach aphids (Myzus persicae Sulz.) were obtained from a colony on Datura plants in the laboratory. The plants were tapped several times to cause most of the aphids that were feeding to withdraw their stylets. After 30 seconds, a number of aphids were gently brushed into a petri plate. The aphids were starved for 30 minutes and then allowed to feed on SMV or TRSV infected tissue. Aphids that were observed with a scanning microscope to be feeding were then picked up with a moistened camel's hair brush and transferred to soybean plants. Care was taken to make sure that the aphids had withdrawn their stylets before they were picked up. One aphid was placed on each of 20 Dare plants. After it was verified that the aphids had fed, the plants were sprayed with insecticide and placed in the greenhouse for observation of symptoms.

Purification

A. Soybean mosaic virus

SMV was partially purified by the method described by Ross (35). Using this technique, freshly harvested SMV-infected soybean

leaves (400 g) were cooled to 5 C and then homogenized in a Waring Blender in twice their weight of cold 0.5 M sodium citrate containing 1% mercaptoethanol. The extract was then strained through cheesecloth to remove the pulp. N-butanol (7 ml/100 ml extract) was added drop by drop with a separatory funnel while the extract was stirred slowly. After curdling was evident, the preparation was placed at 5 C in the refrigerator overnight. The extract was then clarified by subjecting it to a low speed centrifugation (10,000 rpm) in a Servall Centrifuge (Model RC-2) for 10 minutes at 2 C. The greenish-brown supernatant was decanted through glass wool. The liquid was placed in a separatory funnel and allowed to stand at room temperature for 30 minutes at which time the clear amber liquid was drawn off. This liquid was subjected to high speed centrifugation (30,000 rpm) for 90 minutes in the No. 30 rotor of the Beckman Model L-2 ultracentrifuge to pellet the virus. This resulted in clear, slightly greenish pellets. The pellets were rinsed with distilled water and resuspended overnight at 5 C in 2 ml of borate buffer (0.01 M sodium borate, pH 8.3) per tube. The resuspended virus was combined and subjected to another differential centrifugation cycle. At the end of this cycle, the pellets were almost clear. The pellets were again rinsed, resuspended and combined to give a total of 8 ml of virus preparation. The preparation was assayed for infectivity on Dare plants. Ultraviolet absorbances at 260 mu were used to estimate protein concentration. Measurements of the ultraviolet absorption spectra were made on a Perkin-Elmer Model 202 spectrophotometer.

B. Tobacco ringspot virus

TRSV was partially purified following the procedure used by Gooding (17). National Pickling cucumber (Cucumis sativus L.) was used as the production host. The cotyledons were harvested ten days after they were inoculated, fresh weight determined and the tissue frozen. Three hundred grams of frozen tissue were homogenized in a Waring Blender in 300 ml of distilled water containing 1% mercaptoethanol. The sap was filtered through cheesecloth and adjusted to pH 7.2 with a saturated solution of Na_2HPO_4. N-butanol (7 ml/100 ml extract) was added drop by drop as the extract was stirred slowly. The preparation was then clarified at 11,500 rpm for 15 minutes and the resulting supernatant was incubated at 25 C for 12 hours. This was followed by another low speed centrifugation and a high speed sedimentation at 30,000 rpm for 90 minutes. The clear to slightly cloudy pellets produced were resuspended in 0.01 M KH_2PO_4-Na_2HPO_4 at pH 7.2. The preparation was subjected to another cycle of differential centrifugation and the virus was resuspended in buffer to give a final volume of 3 ml. The preparation was assayed for infectivity on Early Ramshorn seedlings. Ultraviolet absorption spectra were used to estimate protein concentration.

Serology

A. Production of antisera

Antisera to TRSV and SMV, respectively, were prepared by injecting partially purified virus preparations into rabbits previously bled for normal serum. The SMV and TRSV preparations contained an estimated 1.0 mg virus/ml and 0.5 mg virus/ml, respectively.

Intramuscular injections were made with virus solutions mixed with Freund's complete adjuvent (Difco Laboratories, Detroit, Michigan). Equal volumes of adjuvent and virus suspension were emulsified using a Sorvall Omni-Mixer. The formation of a thick creamy solution was evidence of complete emulsification.

One half ml of emulsion was injected into each hip of a rabbit and one ml was injected under the skin of the back for each virus, respectively.

Interdermal injections were made by mixing virus suspension with Freund's incomplete adjuvent and injecting 1/4 ml of emulsion into the back foot pads of a rabbit for each virus.

Serum was obtained from the rabbits three weeks after injection.

B. Agar diffusion tests

The Ouchterlony (30) agar double diffusion method was used to test the reactions of SMV and TRSV to several antisera, including that produced in this study. Antisera to TRSV were obtained from J. P. Ross, Plant Pathologist, Crops Research Division, ARS, USDA, Raleigh, North Carolina and H. J. Walters, Professor of Plant Pathology, University of Arkansas, Fayetteville, Arkansas. Antiserum to a North Carolina isolate of SMV was supplied by M. C. Rush, Assistant Professor of Plant Pathology, Louisiana State University, Baton Rouge.

Plates were prepared by pouring approximately 12 1/2 ml of 0.85 Ion Agar No. 2 (Consolidated Laboratories, Chicago Heights, Ill.) containing 0.4 percent sodium azide into nine cm plastic petri dishes. After the agar solidified, a center well was cut in each plate and the agar removed from that well. Four surrounding wells

were cut in the agar 10 mm from the edge of the center well. All wells were cut with a 7 mm cork borer and each had a capacity of approximately 0.2 ml.

Partially purified antigen (SMV or TRSV) was placed in the center well and the various antisera and normal serum in the surrounding wells.

Long flexuous rods such as SMV will not diffuse readily in agar gels. The method described by Purcifull and Shepard (33) for degrading flexuous rod-shaped particles into small antigenically active fragments which diffuse readily in agar gels was followed in these studies. This consisted of mixing equal volumes of SMV preparation and 0.1 M ethanolamine, pH 10.5. The resulting mixture was used in agar diffusion tests.

During formation of precipitin zones, the diffusion plates were kept at room temperature (20-22 C) in a moisture chamber.

C. Microprecipitin tests

Microprecipitin tests under mineral oil were used to test the reaction of SMV to two antisera. Twofold dilutions of SMV and antisera were made in 0.85 percent saline. Equal volumes of virus preparation and antiserum were mixed, covered by a layer of mineral oil and left at room temperature for development of precipitates.

Greenhouse Tests

Six varieties of soybeans were grown in the greenhouse to determine their response to infection with SMV and TRSV, respectively. Varieties used were Bragg, Dare, Davis, Hill, Lee and Semmes. Plants were grown in 12 inch pots, 7 plants per pot, four replications per

variety. Replications were completely randomized. Plants were inoculated when bifoliate leaves were about 3/4 expanded and plants were observed to see that all developed symptoms. Seed were harvested as each variety matured.

To determine the percent seed transmission of SMV and TRSV, seed from virus infected plants were germinated in the greenhouse in flats of sterile soil. Seedlings that developed symptoms were counted and the percent infection was calculated.

Field Tests

To determine the effects of TRSV and SMV infection on the yields of six varieties, plants were inoculated in the field at the University Hill Farm located on the Baton Rouge campus. A superimposed factorial arrangement in a Randomized Block Design was used for each virus. Each plot was four rows wide and 50 feet long; each subplot 25 feet long. A ten foot alley was cut between each subplot to prevent spread of the virus from treated to check subplots. Treatments were assigned to each subplot at random. All treatments were replicated four times. Only the two inner rows of each plot were inoculated, the outer rows serving as guard rows. Each variety was harvested as it matured and seed yield was determined for each treatment.

The analysis of variance was used to analyze field and greenhouse data and a Least Significant Difference (L.S.D.) was used to rank the means.

RESULTS

Host Range Studies

A. Soybean mosaic virus

Forty-four varieties in 22 genera were tested as possible hosts of SMV (Table 1). Of these, only soybean and teaweed (Sesbania exalta (Raf.) Cory) were found to be hosts of the virus. All six varieties of soybeans tested, showed typical SMV symptoms which included vein clearing followed by development of a mosaic pattern on the first trifoliate. Leaflets later developed a typical oak-leaf-appearance and became "blistered" along the main veins on the upper leaf surface. No localized response was produced on soybean; all symptoms were systemic. The six varieties differed little in severity of symptom expression. However, symptoms on Davis were noticeably less severe than those shown by the other varieties. Symptoms on teaweed consisted of small chlorotic to yellow spots similar to local lesions on inoculated leaflets. The virus was systemic in teaweed, but produced no noticeable systemic symptoms.

B. Tobacco ringspot virus

Forty-two varieties in 20 genera were tested as possible hosts of TRSV (Table 1). Twenty-eight varieties or cultivars exhibited a positive response to inoculation with the virus. The cucurbits (watermelon, (Citrullus vulgaris Schrad.), muskmelon (Cucumis melo L.), cucumber (C. sativus L.), pumpkin (Cucurbita pepo L.), and squash (C. pepo L. var. melopepo Alef.)) showed small chlorotic to yellowish

spots on inoculated cotyledons followed by systemic symptoms of very small light yellowish spots on leaves. A systemic mottle was produced on the foliage of Datura. The six soybean varieties, including Lee, Hill, Davis, Semmes, Dare and Bragg, produced rusty flecks on the inoculated bifoliate leaves. Systemic symptoms consisted of a mottle on young trifoliate leaves. All varieties were noticeably stunted, whereas Davis was severely stunted. TRSV on tobacco produced typical ringspots. All varieties of Lima (Phaseolus lunatus L.) and French, or common bean (P. vulgaris L.) produced symptoms in response to inoculation with TRSV. Typical symptoms on beans were brown to purple local lesions on inoculated leaves. As the virus became systemic, the first trifoliates were distorted. Local lesions and flower abortion were symptoms of TRSV on teaweed. Yard long bean (Vigna sesquipedalis L.) and cowpea (V. sinensis Torner) produced necrotic local lesions on inoculated leaves. Systemic symptoms were twisting and distortion of trifoliate leaves. The cowpea variety Early Ramshorn reacted by producing purple local lesions on inoculated leaves, discoloration and necrosis of the stem tip and death of the plant.

Table 1. Host range of SMV and TRSV

Species and variety	Response[1] SMV	TRSV
Arachis hypogaea L. (peanut)	-	-
Capsicum annum L. (pepper) Yolo Wonder	-	-
Cassia obtusifolia L. (coffeeweed)	-	
Citrullus vulgaris Schrad. (watermelon) Charleston Gray	-	+
Clitoria mariana L. (butterfly pea)	-	
Cucumis melo L. (muskmelon) Hale's Best		+
C. sativus L. (cucumber) National Pickling	-	+
Cucurbita pepo L. (pumpkin) Small Sugar		+
C. pepo L. var. melopepo Alef. (squash) Yellow Straightneck		+
Datura stramonium L. (jimson weed)		+
Desmanthus illinoensis (Michaux) MacM.	-	
Desmodium sp. L. (beggar's tick)	-	-
Glycine max (L.) Merr. (soybean)		
Bragg	+	+
Dare	+	+
Davis	+	+
Hill	+	+
Lee	+	+
Semmes	+	+
Helianthus annus L. (sunflower) Mammoth Russian	-	-
Lathyrus odoratus L. (sweet pea)	-	

Table 1 . . . continued

Species and variety	Response SMV	Response TRSV
Lupinus sp. L. (lupine)	-	
Lycopersicon esculentum Mill. (tomato) Bonny Best	-	-
Medicago sativum L. (alfalfa)	-	-
Nicotiana tabacum L. (tobacco) Havanna-425	-	+
Pisum sativum L. (garden pea) Laitonian	-	-
P. sativum L. var. arvense (Austrian winter pea)	-	-
Phaseolus coccineus L. (scarlet runner bean)	-	
P. lunatus L. (Lima beans)		
Carolina Sieva Pole Lima	-	+
Henderson Bush Lima	-	+
Jackson Wonder Bush Lima	-	
Willow Leaf Pole Lima	-	+
P. vulgaris L. (French bean)		
Contender Bush Bean	-	+
Bountiful Bush Bean	-	+
Great Northern Bean	-	+
Harvester Bush Bean	-	+
Kentucky Wonder Wax Pole Bean	-	+
Pinto Bean	-	+
Red Kidney Bean	-	+
Physalis sp. L. (ground cherry)		-
Sesbania exalta (Raf.) Cory (teaweed)	+	+
Sorghum vulgare Pers. (sorghum)	-	-
Trifolium incarnatum L. (crimson clover)	-	-

Table 1 . . . continued

Species and variety	Response SMV	Response TRSV
T. repens L. (white Dutch clover)	-	-
Vicia angustifolia Reichard (vetch) Narrow Leaf Vetch	-	-
V. faba L. (broad bean)	-	-
Vigna sesquipedalis L. (yard long bean)	-	+
V. sinensis Torner (cowpea)		
Clay	-	+
Early Ramshorn	-	+
Silver Skin Crowder	-	+
Wisteria sinensis (Sims) Sweet (wisteria)	-	

[1] No sign indicates not tested, a plus sign(+) indicates a positive response and a minus sign(-) indicates no response.

Influence of Temperature on Symptom Expression

SMV symptoms were much more severe at 20 C than at temperatures of 30 C or above. At 20 C leaflets showed a strong mosaic pattern and became severely distorted and blistered. Only half of the plants inoculated produced visible symptoms at 40 C and those that were produced were very mild.

TRSV failed to develop visible symptoms at 40 C. Plants grown at 20 C exhibited very severe symptoms, but those grown at 25 C produced milder symptoms. Symptoms at 20 C included a rusty flecking, yellowing and puckering of emerging terminal leaves and bronzing of the stem tips which are typical bud blight symptoms.

Physical Properties

A. Dilution end point

The dilution end point of SMV in extracted sap was 10^{-4}. No plants were infected with dilutions 10^{-5} or greater. TRSV was not infectious when diluted beyond 10^{-3}.

B. Thermal inactivation point

SMV survived ten minutes at 60 C, but not ten minutes at 65 C. TRSV was inactivated at temperatures between 55 and 60 C.

C. Longevity *in vitro*

SMV survived *in vitro* one day at room temperatures. TRSV was inactivated after three days at room temperatures.

Insect Transmission

C. trifurcata (bean leaf beetle) failed to transmit either SMV or TRSV from infected to healthy Dare soybean plants. *M. persicae* (green peach aphid) failed to transmit TRSV from infected to healthy Dare soybeans, but did transmit SMV in similar tests. Typical SMV symptoms developed on three of the 20 plants on which viruliferous aphids were allowed to feed. No symptoms developed on any of the 20 control plants fed on by virus-free aphids.

Seed Transmission

Seeds harvested from healthy and virus-infected plants grown in the greenhouse were planted to determine the percentage of seed transmission in the six varieties. SMV infection was transmitted highest in Dare, Hill and Semmes and low in Lee, Davis and Bragg

(Table 2). TRSV transmission was high in all varieties and extremely so in Hill, Semmes, Davis and Lee.

Table 2. The percentage of SMV and TRSV-infected seedlings grown from seeds obtained from SMV and TRSV-infected plants and controls.

Variety	SMV		TRSV		Control	
	No. Infected Seedlings[1]	%	No. Infected Seedlings	%	No. Infected Seedlings	%
Lee	13/182	7	100/149	67	0/140	0
Davis	3/135	2	95/133	71	0/132	0
Bragg	7/130	5	41/137	30	0/137	0
Dare	37/120	30	45/131	34	0/129	0
Hill	22/132	22	110/118	93	0/147	0
Semmes	30/125	24	95/126	75	0/142	0

[1] Denominator, total number of seedlings; numerator, number of seedlings infected.

Purification

Spectrophotometric analysis of the final SMV preparation yielded an estimated concentration of one mg of virus per ml of suspension and produced 2 mg of virus per 100 g of infected tissue. Prominent features of the ultraviolet absorption spectrum of the purified virus were a minimum at 240 mu and a maximum between 255 and 260 mu (Figure 1).

Final virus concentration of the TRSV preparation was 0.5 mg of virus per ml of suspension and yield was 0.5 mg of virus per 100 grams of infected tissue. The ultraviolet absorption spectrum showed a minimum at 245 mu and a maximum between 260 and 265 mu (Figure 2).

Both SMV and TRSV preparations were infective when tested on Dare soybeans and Early Ramshorn cowpea, respectively.

Serology

SMV failed to react with antisera in either microprecipitin or agar diffusion tests. TRSV formed distinct precipitin zones when allowed to react with antisera to Louisiana, Arkansas and North Carolina isolates of the virus (Figure 3).

Greenhouse Tests

The bean yields of SMV-infected Dare, Hill, Semmes, Lee, Bragg and Davis plants were compared with yields of similar healthy control plants (Table 3). The average mean yields of all infected varieties except Davis were less than the average mean yields of corresponding controls. An analysis of variance revealed a highly significant difference in yields between virus infected plants and controls. There was also a significant interaction between the varieties and the virus treatments. This indicated that different varieties reacted differently to inoculation with SMV. A Least Significant Difference (L.S.D.) was then used to determine in which varieties the yields were significantly reduced by the virus infection. There was a highly significant yield reduction of Lee, Hill and Semmes. Yield reduction of Dare closely approached significance. The yields of Bragg and Davis were not significantly reduced by the virus treatment.

Table 3. The average mean yields in grams of soybeans from healthy and SMV infected plants grown in the greenhouse.

Variety	Healthy Control Plants	SMV-infected plants	Difference
Lee	93.08	62.95	30.13
Davis	44.32	56.40	-12.08
Bragg	59.78	49.68	10.10
Dare	71.22	52.50	18.72
Hill	77.02	47.12	29.90
Semmes	59.00	32.48	26.52

L.S.D. .05=19.78
.01=26.64

As shown in Table 4, the yields of all six soybean varieties were greatly reduced by TRSV. According to an analysis of variance, there was a highly significant difference in yields between the virus infected plants and the control plants. There was not a significant interaction between varieties and virus treatments; therefore, there was a highly significant reduction in yields of all varieties.

Table 4. The average mean yields in grams of soybeans from healthy and TRSV-infected plants grown in the greenhouse.

Variety	Healthy control plants	TRSV-infected plants	Difference
Lee	69.45	22.50	46.95
Davis	71.32	16.27	55.05
Bragg	78.57	20.52	58.05
Dare	80.65	34.20	46.45
Hill	70.12	27.47	42.65
Semmes	78.17	23.57	54.60

L.S.D. .05=17.10
.01=23.07

Field Tests

SMV symptoms in the field were not severe and became less distinct as the plants grew older. Hill and Dare exhibited more severe symptoms than the other varieties. Typical symptoms in late July consisted of an occasional leaf showing blistering on the upper surface and a slight downward curling of the leaf margins.

Typical TRSV symptoms observed on young plants were a yellowing and flecking of young leaves and severe stunting of the young plants. Somewhat later, symptoms became less apparent and the plants appeared to recover. At maturity, however, there was a readily apparent difference in size between infected and healthy control plants. All varieties inoculated with TRSV remained green after the control plants had shed their leaves.

Yields of SMV-inoculated plants were compared with yields of control plants in 1969 and 1970 (Table 5). An analysis of variance for each year's results revealed no significant differences in yields between varieties treated with SMV and control varieties. However, except for Lee in 1969, all differences between average mean yields of treated and control plants were in the same direction; that is, control yields were greater than treatment yields.

Table 5. The average mean yields in pounds of soybeans from healthy and SMV-inoculated plants grown at the University Hill Farm in Baton Rouge during 1969 and 1970.

Variety	Control plants		SMV-inoculated plants		Difference	
	1969	1970	1969	1970	1969	1970
Lee	7.00	7.60	7.58	7.00	-0.58	0.60
Davis	9.62	13.40	9.55	12.20	0.07	1.20
Bragg	9.85	7.50	9.22	6.80	0.63	0.70
Dare	9.35	10.20	7.45	8.80	1.88	1.40
Hill	6.82	6.10	5.75	5.80	1.07	0.30
Semmes	7.90	6.80	7.68	6.60	0.22	0.20

Yields of all TRSV-inoculated varieties were apparently reduced in comparison to yields of control varieties (Table 6). According to an analysis of variance, there was a significant difference in yields between virus treated varieties and controls. There was also a highly significant interaction between the varieties and their reaction to TRSV. The L.S.D. test indicated that there was a highly significant

reduction in yields of Davis soybeans due to TRSV. There was a significant reduction in yields of the Semmes variety. Reductions in yields of all other varieties were not statistically significant.

Table 6. The average mean yields in pounds of soybeans from healthy and TRSV-inoculated plants grown at the University Hill Farm in Baton Rouge during 1970.

Variety	Control plants	TRSV-inoculated plants	Difference
Lee	7.4	4.6	2.8
Davis	12.5	6.6	5.9
Bragg	6.4	4.6	1.8
Dare	9.3	7.7	1.6
Hill	6.2	4.0	2.2
Semmes	6.8	2.7	4.1

L.S.D. .05=3.04
.01=4.21

DISCUSSION

This research produced information concerning the symptomatology, host range, physical properties, methods of transmission and varietal reactions of hosts of SMV and TRSV. Aside from their value in identification of the viruses, the study of these characters has a practical meaning because they provide information which may lead to eventual control of these diseases in soybeans.

One interesting characteristic of SMV is its narrow host range. This was confirmed in the host range studies (Table 1) in which only two species of the 22 genera tested became infected with the virus. Both soybean and teaweed have previously been reported as systemic hosts for SMV (15). Henderson Bush Lima bean has been reported as a systemic host for SMV by Walters (52) who obtained a latent to mild infection. Ross (34) reported Kentucky Wonder Pole Wax bean as a local lesion host for SMV. Neither variety became infected with SMV in this study. It is possible that the Louisiana isolate used in this study differed from the Arkansas and North Carolina isolates in its pathogenicity to these two bean varieties. This has been shown to occur by Ross (37).

SMV symptoms observed on the six soybean varieties were similar to those reported in the literature (6, 7, 16, 25). Temperature had a definite influence on symptom expression in plants infected with SMV. A decrease in severity of symptoms was observed as the temperature increased. This was consistent with temperature effects observed by Conover (7) and Walters (52).

The broad host range of TRSV was demonstrated by the observation that 28 varieties out of 42 tested became infected with the virus. All of the hosts listed (Table 1) have previously been reported as hosts of TRSV (41).

Symptoms produced on soybeans were similar to those described for TRSV by Allington (1) and Hilderbrand and Koch (18). Expression of symptoms on TRSV-infected plants was enhanced by low temperatures. This was shown by comparing severe bud blight symptoms on plants grown at 20 C with mild symptoms on plants grown at 25 C. Similar results were reported by Khan and Latterall (26).

The dilution end point of SMV was 10^{-4} which falls within the range of 10^{-3}-10^{-5} previously reported (15). The thermal inactivation point was between 60 and 65 C which agreed with results obtained by Galvez (15). SMV survived in vitro for one day which agrees with 1-4 days survival shown by previous workers (15, 41).

The dilution end point of 10^{-3} for TRSV was the same as that reported by Smith (41). The thermal inactivation point between 55 and 60 C agrees with information reported by Smith (41). Survival in vitro of only three days was less than the 5-6 days reported by previous workers (31, 41). No possible cause of this difference was observed.

SMV and TRSV were both easily transmitted mechanically as has been shown by all previous investigators. No insect vector was found for TRSV, but SMV was readily transmitted by the green peach aphid which has been found to be an efficient vector of this virus (15). Green peach aphids were present in Louisiana during April, May

and early June in large populations on soybeans (20). Aphids are probably the most important vectors of SMV in Louisiana.

Both SMV and TRSV were readily transmitted through soybean seed. Percentages of SMV seed transmission obtained (Table 2) agree closely with results obtained by Horn et al. (20) who also state that SMV infection in commercial seed lots of Dare and Hill are often high. The high percentage of TRSV transmission (Table 2) is not unusual. Althow (3) demonstrated 100 percent transmission of TRSV in some soybean varieties.

The ultraviolet absorption spectra of SMV and TRSV (Figures 1 and 2, respectively) are typical of purified nucleoprotein preparations which usually show a maximum absorption around 260 mu and a minimum absorption at 240 mu (4). Symptoms produced when preparations were assayed for infectivity indicated that an infectious agent had been purified in each case.

SMV did not react against any antiserum, including its own, in either the microprecipitin tests or the agar diffusion tests. TRSV reacted against all of the antisera, including its own, and formed three distinct precipitin zones (Figure 3). This confirmed that the virus isolate was TRSV.

In the 1969 and 1970 field tests, no significant differences in yields of soybeans were obtained between the SMV-inoculated plants and the controls. Except for Lee in 1969, all inoculated varieties did show a slight reduction in yields when compared to controls (Table 5). This is in sharp contrast to results obtained in greenhouse studies where Lee, Hill and Semmes showed highly significant reductions in yield and Dare closely approached a significant reduction

at the .05 level (Table 3). Reduction in yields of Bragg and Davis were not significant although the yields of Bragg were reduced about 17 percent. Davis was the most resistant of the six varieties.

The fact that significant reductions were obtained in the greenhouse and not in the field may be due to the percent of infection obtained in the greenhouse as opposed to the field. Plants in the field were closely spaced, which made inoculation very difficult. Although no effort was made to determine the percent of infection in the field after inoculation, it must have been rather low because the yields of the infected plants were far greater than were anticipated. All inoculated plants in greenhouse tests were observed to be infected.

The data presented in Table 5 show that there was a highly significant reduction in yields of all varieties inoculated with TRSV in the greenhouse. This closely agreed with the data of Crittenden et al. (9) who reported yield losses of approximately 60 percent when only 85 percent of the plants were infected. Field data indicate that all varieties did not react alike when infected with TRSV under field conditions (Table 6). Although the yields of all inoculated varieties were less than the controls, only Davis and Semmes were reduced significantly.

Field and greenhouse results did not agree in regard to significance of yield reduction. There is no reason to believe that the TRSV plots had a higher percent infection that SMV plots; therefore, it was again speculated that higher yields in the field reflected less than 100 percent infection.

SUMMARY

1. Two Louisiana virus isolates were identified by symptomatology, host range, physical properties, transmission and serology as soybean mosaic virus (SMV) and tobacco ringspot virus (TRSV).
2. Temperatures had a marked influence on the intensity of the symptoms described on soybeans. At 20 C, symptoms were more severe in test plants than in plants grown at 25 C or higher.
3. SMV was transmitted in 30 percent of the seed of Dare soybeans and to lesser extents in all other varieties. TRSV was transmitted at a high rate in all varieties and as much as 93 percent in Hill.
4. In greenhouse tests where all inoculated plants were observed to be infected, SMV significantly reduced yields of Lee, Dare, Hill and Semmes varieties. Davis was the most resistant variety to SMV. TRSV significantly reduced yields of all varieties tested.
5. Under field conditions where the extent of infection was not known, the yields of all varieties were not significantly reduced by SMV. The yields of the varieties Davis and Semmes were significantly reduced by TRSV.
6. SMV failed to react with antisera in either microprecipitin tests or agar diffusion tests. TRSV formed distinct precipitin zones when allowed to react with antisera to Louisiana, Arkansas and North Carolina isolates of the virus.

7. Ceratoma trifurcata (bean leaf beetle) failed to transmit either SMV or TRSV from infected to healthy Dare soybean plants. Myzus persicae (green peach aphid) transmitted SMV from infected to healthy Dare plants, but did not transmit TRSV in similar tests.

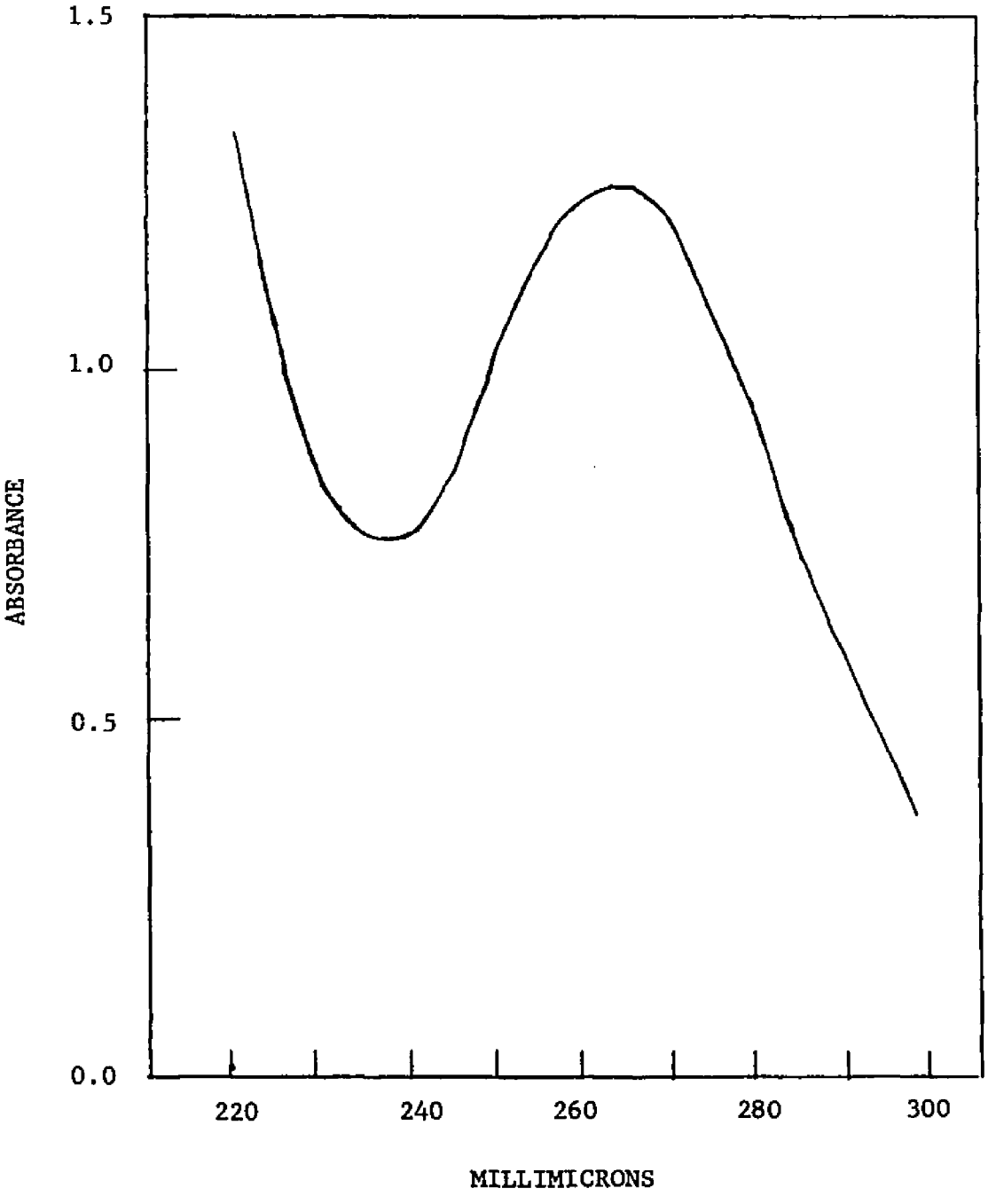

Figure 1. Absorption spectrum of a purified preparation of the Louisiana SMV isolate.

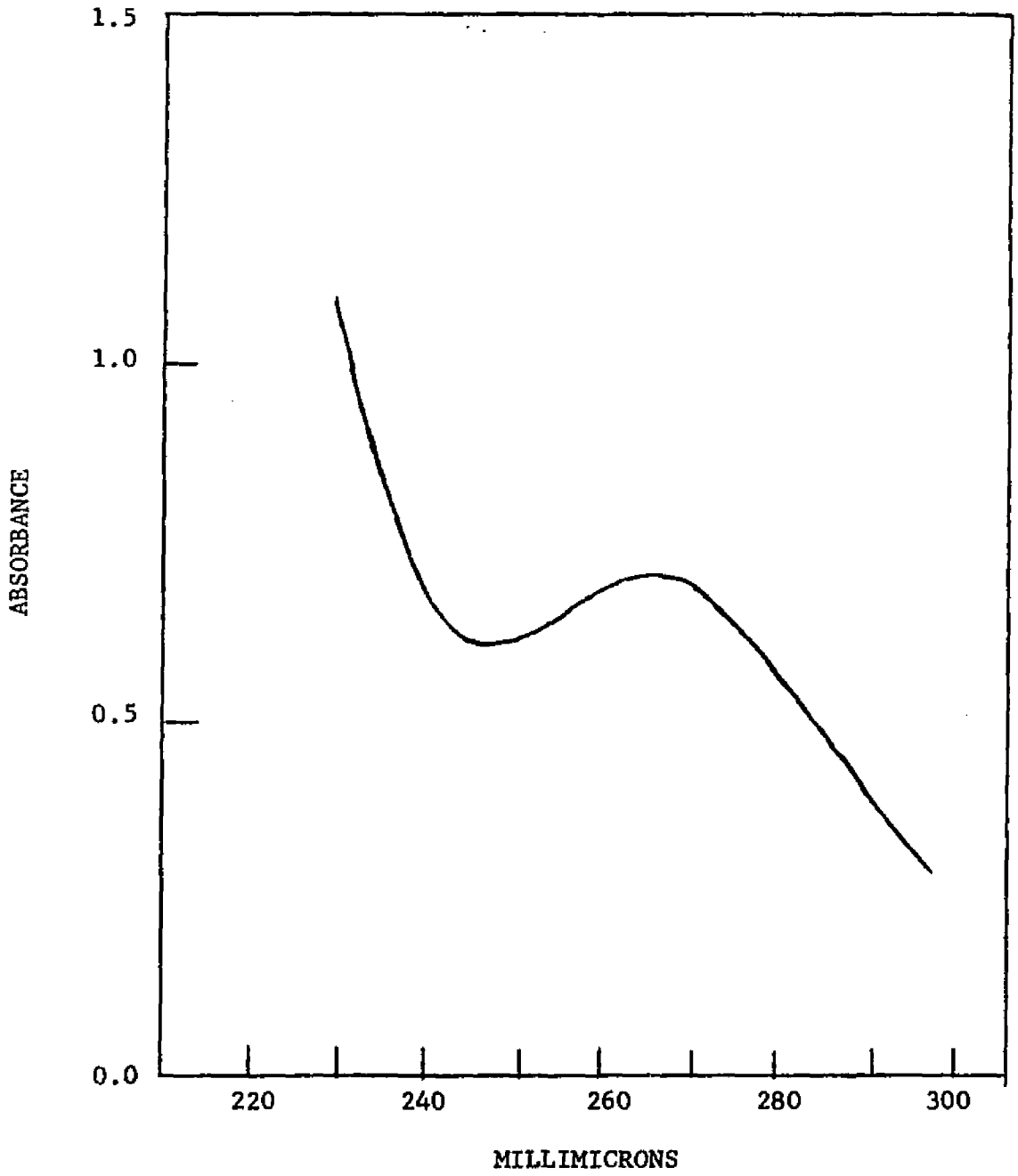

Figure 2. Absorption spectrum of a purified preparation of the Louisiana TRSV isolate.

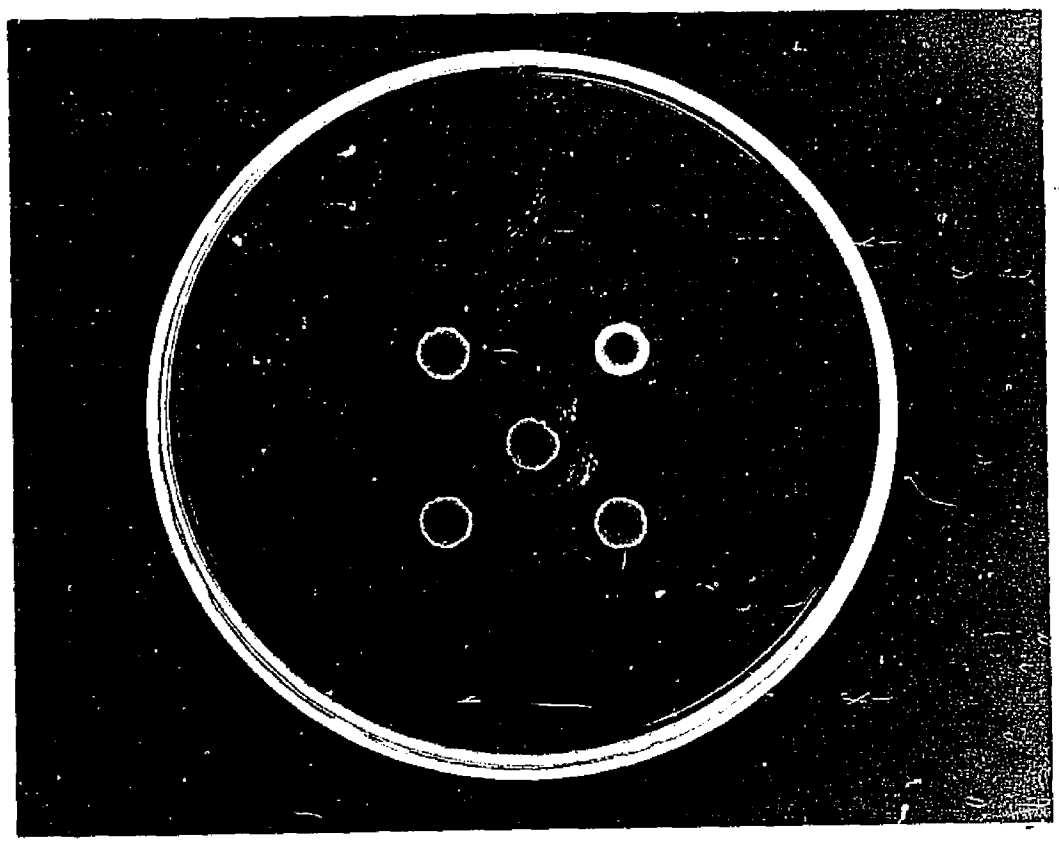

Figure 3. Agar diffusion plate showing precipitin zones between a purified preparation of the Louisiana TRSV isolate (center well) and antisera to TRSV isolates from (A) Louisiana, (B) North Carolina and (C) Arkansas. Well D contained distilled water.

Figure 4. Trifoliate leaf of a healthy Dare plant.

Figure 5. Trifoliate leaf of an SMV-infected Dare plant showing rolling and curling of the leaf margins giving a typical oak-leaf-appearance.

Figure 6. Trifoliate leaf of an SMV-infected Dare plant showing blistering (arrow) on the upper leaf surface.

Figure 7. Dare seedling inoculated with TRSV and grown at 20 C showing typical bud blight symptoms.

Figure 8. Bifoliate leaf of Early Ramshorn cowpea showing necrotic local lesions caused by TRSV.

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VITA

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EXAMINATION AND THESIS REPORT

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Major Field: Plant Pathology

Title of Thesis: Identification and Varietal Response of Soybeans to Soybean Mosaic Virus and Tobacco Ringspot Virus

Approved:

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Date of Examination:

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